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ENRICHED ROTIFERS VERSUS COPEPODS AS LIVE PREY FOR ATLANTIC BLUEFIN TUNA (*THUNNUS THYNNUS* L.) LARVAE

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There are still many issues that need to be investigated and solved in the rearing of larval and juvenile Atlantic bluefin tuna (*Thunnus thynnus*; ABT) to prevent "mass-mortality" during these early developmental stages. Initial data related to the feeding sequence of ABT larvae suggested that mortality observed during the first stages of life could be partly due to nutritional deficiencies. Our overarching aim is to gain knowledge to better understand the molecular basis of ABT larval nutrition during first feeding. In order to achieve this goal, the effects of four enrichment protocols for rotifer *B. rotundiformis* as well as copepod nauplii on survival, growth performance and development, and expression of key genes of lipid metabolism, antioxidant defence, and digestion were investigated in ABT larvae at the onset of exogenous feeding. Growth performance was evaluated by biometry (total length and total dry mass), and development by notochord flexion index, as well as the expression of myosin heavy chain (myhc) and tropomyosin (tropo) genes, both implicated in myogenesis. Key genes involved in major lipid pathways including fatty acid and long-chain polyunsaturated fatty acid (LC-PUFA) biosynthesis (fas, fads2, and elovl5), lipid transport (fabp2, 4, and 7), deposition (lpl), and β-oxidation (cpt1 and aco), and their control and regulation (transcription factors ppara, pparγ, lxr, rxr, srebp1, and srebp2), were evaluated in ABT larvae fed the different live prey. Additionally, ABT antioxidant protection status was evaluated by determining the expression of genes encoding antioxidant enzymes superoxide dismutase (sod), catalase (cat), and glutathione peroxidase (gpx1 and gpx4). Digestive capabilities were assessed by the relative expression of the genes coding for the enzymes trypsin (tryp), aminopeptidase (anpep), alkaline phosphatase (alp), amylase (amy), pancreatic lipase (pl), bile salt-activated-lipases (bal1 and bal2), and phospholipase A_2 (pla2).

ABT larvae were fed enriched rotifers *B. rotundiformis* and copepod nauplii *Acartia tonsa* from first feeding to 15 days post-hatching. Rotifers were enriched with different commercial products: Origreen Skretting® (OG), Multigain Biomar® (MG), Algamac 3050® (AG), and Red Algamac® (RA) plus selenium and vitamin E. Copepods (COP) were cultured with the algae *Rhodomonas salina*.

Growth and development parameters and high expression of myogenesis genes myhc2 and tropo indicated that COP were superior to enriched rotifers as live prey for first feeding ABT. Larvae fed COP and AG-rotifers showed lower expression of the transcription factors, pparγ and srebp2. The expression of fas showed differences among treatments, with highest relative expression in larvae fed COP and RA-rotifers. In relation to fatty acid catabolism, larvae fed RA-rotifers showed the highest expression of aco, with lowest expression observed in larvae fed COP. The expression profiles of lipid homeostasis genes showed that larvae fed COP showed high expression of fabp2 and fabbp4. Larvae fed AG-rotifers showed the lowest level of lpl expression, with highest expression observed in larvae fed OG-rotifers. Regarding antioxidant enzyme gene expression, sod showed high values in larvae fed COP and RA-rotifers, with larvae fed MG-rotifers showing a lower expression level. A similar pattern was observed for the expression of cat, gpx1 and gpx4. The expression of genes for digestive enzymes showed that tryp expression level was highest in COP-fed larvae but, in contrast, these larvae showed the lowest expression levels of anpep and alp. ABT larvae fed AG-rotifers displayed the lowest expression level of pla2. Expression of bal1 and bal2 presented a similar pattern, with highest values in ABT fed COP and lowest expression in larvae fed AG-rotifers.

In conclusion, and in agreement with our previous trial, the present study showed that copepods were a superior live prey for first feeding ABT larvae compared to enriched rotifers, as indicated by the higher growth and flexion index achieved by larvae fed COP. All dietary treatments in the present study appeared to satisfy LC-PUFA requirements as no significant effects were observed in the expression of the genes related to the homeostasis and biosynthesis of these compounds (pparα, srebp1, lxr, rxr, fads2d6, and elovl5). Addition of organic selenium and α-tocopherol did not enhance larval performance, probably indicating that the levels contained in copepods ($0.4 \mu g.g^{-1}$ and $170 mg.kg^{-1}$, respectively) were sufficient to fulfil nutritional requirements.